The London Cord Blood Bank: analysis of banking and transplantation outcome

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Summary

Cord blood units ($n = 5500$) stored at the London Cord Blood Bank, including 59 units transplanted into a high risk and heterogeneous group of patients, were analysed. Transplant outcome data was available for 44 patients with a median clinical follow-up of 14 months (range 3–44 months). Over 40% of the collected units were of ethnic minority origin with a median volume of 79 ml (range 40–240 ml) and a median total nucleated cell (TNC) count of $11·9 \times 10^9$/l (range $10·0–24·8 \times 10^9$/l). The average patient's weight was 28 kg (range 5–80 kg) and the median age was 8 years (range 0·7–40 years). The median number of nucleated cells infused was 4×10^7/kg (range $1·10–16 \times 10^7$/kg). Neutrophil engraftment of $0·5 \times 10^9$/l was observed in 33 (74±%) patients with an average time of 28 days (range 11–60). The Kaplan-Meier estimate of acute graft-versus-host disease (grade II >) at day 100 was 37 ± 7% and in 27 (62%) patients, it was grade I or absent. The overall survival and disease-free survival at 2 years was 49 ± 8% and 41 ± 8%, respectively. Two years after transplantation the survival rate was 69% and 54% for patients receiving a 6/6 or 5/6 HLA matched units, respectively. Infection was the main cause of transplanted related mortality in these patients.

Keywords: cord blood banking, transplantation, clinical outcome, haematopoietic stem cells, cord blood stem cells.

The first successful umbilical cord blood (CB) transplant was performed in 1988 in a patient with Fanconi's anaemia using CB from a human leucocyte antigen (HLA)-identical sibling (Gluckman *et al*, 1989), demonstrating that a single CB unit contained a sufficient number of stem cells for haematopoietic reconstitution. Since then, over 2500 patients worldwide have been transplanted using CB to treat a variety of conditions including haematological malignancies, bone marrow failures and congenital immunodeficiencies (Rubinstein *et al*, 1998; Gluckman, 2000; Gluckman & Locatelli, 2000; Gluckman *et al*, 2001).

This increased usage of CB stem cells has led to the collection and storage of frozen HLA-typed units by a number of CB banks that have been established over recent years (Gluckman *et al*, 1998; Navarrete *et al*, 1998a; Kato *et al*, 2000; Stanworth *et al*, 2001). These units can be searched via the international CB and bone marrow donor registries, including Netcord (http://www.netcord.org) and Bone Marrow Donors Worldwide (BMDW; http://www.bmdw.org), where over 1 50 000 CB units are now registered. Once a suitable match

has been identified, a CB unit can be made available to a transplant centre within a matter of days. This is of obvious benefit to the patient who requires an urgent transplant and cannot wait for weeks or months to procure bone marrow from a suitable donor. Other advantages of CB include the reduced risk of viral transfer; for example the majority of CB donations are free from CMV infection, which can cause significant post-transplant complications (Nichols *et al*, 2002). In addition, clinical data indicate that there is a reduced risk of graft-versus-host disease (GVHD) following CB transplantation compared with bone marrow, allowing a greater degree of HLA disparity between donor and recipient to be acceptable (Rocha *et al*, 2000; Thomson *et al*, 2000; Barker *et al*, 2001; Laughlin *et al*, 2001; Rocha *et al*, 2001; Wagner *et al*, 2002).

The London Cord Blood Bank (LCBB) has been collecting units since February 1996 from two local London hospitals with a high proportion of patients from ethnic minority groups not adequately represented in the United Kingdom bone marrow donor registries. (Armitage *et al*, 1999a; Brown *et al*, 2000). To date (July 2003), the LCBB has collected and

stored over 5500 units of which 59 have been issued for transplantation to patients worldwide.

In this report, we summarize the procedures for collection, processing and testing of CB units stored at the LCBB, including an update on those banked and issued for transplantation. In addition, we present a follow-up analysis of the outcome of these transplants, in order to assess the efficacy of CB units provided by a single centre. Sufficient clinical follow-up data of at least 3 months post-transplant were obtained from 44 patients.

Patients and methods

Collection, processing and cryopreservation

Cord Blood was collected from freshly delivered placentae at hospitals where local research ethics committee approval has been granted. Collection of the CB was overseen by technical agreements between the hospitals' obstetric department, clarifying the distribution of responsibilities and outlining expectations. Trained LCBB staff, based at the delivery suite, harvested the CB *ex utero* by suspending the placenta and cannulating the vein, allowing blood to drain by gravity into a Stemflex umbilical CB collection bag (MacoPharma UK Ltd, Twickenham, Middlesex, UK). Units containing 40 ml or more blood were processed within 24 h of collection.

All processing stages were performed either in a closed system or in an environmentally controlled cleanroom. CB units were reduced to a standard volume of 21 ml prior to freezing using the 'top and bottom Optipress II method' as previously described (Armitage *et al*, 1999b), removing excess plasma and red cells to leave the required cells in the buffy coat component. A 50% solution of dimethyl sulphoxide diluted in Dextran 40 was added to the cells to give a 10% final concentration. The units were then vacuum packed into an overwrap bag, prior to controlled rate freezing and storage in LN_2.

Consent for storage was obtained from the mother within 24 h of collection. Those units for which consent was not obtained were removed from the bank and discarded. Other reasons for disposal included: a total nucleated cell count of $<4 \times 10^8$ after processing, nucleated cell recovery after processing of <70%, nucleated cell viability of <80%, repeat reactive results for mandatory infectious disease markers, abnormal blood film report and exclusion of the mother because of medical or behavioural risk criteria (Navarrete & Armitage, 2002).

Testing of CB and maternal samples

A total of 4 ml of CB were removed from the collection bag for a full blood count (FBC) and HLA typing. In addition, a blood film was prepared from each unit on which a manual nucleated red cell count and visual assessment of the blood cells was performed. All other tests on the CB unit utilized residual plasma and red cells obtained as a result of the processing, in order to minimize loss of stem cells. Units were typed for ABO, Rh and Kell. They were also screened for the mandatory microbiology markers as stipulated for UK blood donors, currently including human immunodeficiency virus (HIV), hepatitis C and B viruses (HCV, HBV), human T-lymphotropic virus (HTLV), cytomegalovirus (CMV) and syphilis. Viability of the cells, FBC and CD34 counts were determined on 0·3 ml of the final product. After addition of the cryoprotectant, samples were removed and screened for aerobic and anaerobic bacteria and for fungal contamination.

Maternal blood samples were taken on the postnatal ward, during the interview undertaken by the LCBB collection staff. The interview included steps for obtaining informed consent, details of the mother's behavioural, travel history and ethnic origin. A full medical history was obtained 8–10 weeks after donation using a telephone questionnaire taken by trained staff. The questionnaire was regularly updated to reflect as closely as possible the current National Blood Service donor selection criteria plus additional criteria relevant to CB and transplantation (Warwick & Barbara, 2000). Similarly to CB, the mother's sample was tested for the all mandatory microbiological markers. All information obtained on the CB unit and maternal samples was collated and reviewed before proceeding with the HLA typing. The majority of newborn donors, from our collection hospitals, underwent haemoglobinopathy screening as a routine by the Regional Haemoglobinopathy Screening Service and the results were provided to the LCBB. These results would be of particular relevance for those units from the ethnic groups targeted for recruitment.

Throughout processing, a series of samples were removed and archived for future testing that may be required at the time of issue, e.g. newly introduced or more sensitive mandatory microbiology tests, viability assessment of the product at time of issue or other tests as requested by the transplant centre. These samples included plasma, viable cells and material suitable for the isolation of DNA, such as a sample of umbilical cord tissue taken at the time of collection. Plasma and cells for DNA extraction were also archived from the mother.

HLA typing

The HLA typing of the units was performed on DNA obtained from 3·5 ml of the 4-ml CB sample removed. Genomic DNA was extracted using an in-house salting-out technique. If additional DNA was required, a section of umbilical cord tissue was processed using the Nucleon™ extraction method. HLA typing was performed by polymerase chain reaction and sequence-specific oligonucleotide probes (PCR-SSOP) (Dynal RELI™), PCR and sequence-specific primers (PCR-SSP) or sequencing based typing (SBT). All units were initially typed for HLA-A, B and DRB at low to medium resolution (Navarrete *et al*, 1998b). Further testing of additional HLA loci or high-resolution level definition was performed on the request of the transplant centre. Stored maternal DNA was

HLA-typed for quality control and to determine the HLA type of the unit, if required. Otherwise, whenever a request was received for confirmatory typing of a CB unit that was to be considered as a candidate for transplantation, the HLA typing of the mother was performed in order to confirm that it shared one HLA haplotype with the CB unit.

Registration, selection and issue of CB units

Details of the units, including the HLA type and the nucleated cell content, were registered with Netcord and with the British Bone Marrow Registry (BBMR), which then submitted them to BMDW. Prior to issue, the CB and the respective maternal sample were routinely tested for HIV, HCV and CMV by PCR, in addition to hepatitis B core antigen (HBc) and HTLV antibodies, where previously not tested, and any microbiology markers that were mandatory for blood donors at the time of issue. The stored blood film was re-examined by a paediatric transplant consultant and the local Cytogenetics Malformation Register was contacted to enquire whether the infant had, subsequent to donation, been investigated for a chromosomal abnormality or been registered as having a malformation not recognized at the time of donation. Internal quality checks also included a viability assessment, colony-forming unit (CFU) analysis and confirmatory HLA typing. In addition, DNA extracted from the integrally attached bleed line of the unit was compared with DNA used for HLA typing by analysing short tandem repeat (STR) markers. Amplified products from six STR loci (VWA31, TH01, F13A1, FES/FPS, D21S11 and FGA), in addition to the X-Y homologous gene, were run on an ABI PRISM® 310 Genetic Analyser (Applied Biosystems, Warrington, UK) under non-denaturing conditions. Mobility values were then examined to ensure identical STR profiles (Patel *et al*, 2003).

Clinical endpoints definition

Engraftment. Neutrophil engraftment was defined as the first of three consecutive days when the absolute neutrophil count (ANC) was $\geq 0.5 \times 10^9/l$ with evidence of donor haematopoiesis. Patients in whom no engraftment occurred were censored if they died before day 60, as were patients receiving a second transplant for non-engraftment.

Acute and chronic GVHD. Patients were considered at risk of grade II-IV acute GVHD at day +1, whereas only those with sustained engraftment of donor haematopoiesis and surviving for more than 100 days after transplant were evaluated for the signs and symptoms of chronic GVHD. Acute and chronic GVHD were evaluated according to previously defined criteria (Glucksberg *et al*, 1974; Shulman *et al*, 1980).

Overall survival. Overall survival was defined as the time between transplantation and death as a result of any cause. Data on patients were censored at the time of the last follow-up visit.

Disease-free survival. Disease-free survival was defined as the time between transplantation and death or relapse for malignant disorders or late graft failure (or autologous transplantation) for non-malignant diseases. Data on patients were censored at the time of the last follow-up visit.

Statistical analysis

The reference date of the analysis was July 1, 2003. Time to acute and chronic GVHD, neutrophil engraftment and overall survival were estimated by the Kaplan-Meier method. Univariate prognostic analyses were performed for neutrophil recovery and survival and used the log-rank test, testing the influence on each end point of diagnosis (malignancies versus non malignancies), recipient and donor gender and identity, recipient age (children <15 years versus adults ≥15 years), recipient CMV serology (negative versus positive), recipient weight (≥ or <30 kg), ABO compatibility, number of nucleated cells infused (> or $<3.7 \times 10^7$/kg of recipient's weight) and number of HLA disparities (defined as disparities at the specificity level for HLA-A and -B and at the allele level for HLA-DRB1). All variables associated with outcome with a *P*-value less than 0·10 in univariate analysis were included in a Cox proportional hazard model. Then, a backward stepwise procedure was used to select covariates (*P* < 0·05) included in the final Cox proportional hazard model. Because of the small number and heterogeneity of patients, a multivariate analysis was performed only for survival. Statistical analysis used the SPSS computer program (SPSS Inc., Chicago, IL, USA).

Results

Collection of CB units

Over 5500 donations have been successfully banked, the volumes and cell count characteristics of which are shown in Table I. The median blood volume collected was 71·9 ml (range 40–240·2 ml) with a median total nucleated cell recovery of $11.9 \times 10^9/l$ (range $10.0-24.8 \times 10^9/l$). CD34 data was only available on 1654 (29·1%) of units banked, with a median count of $0.03 \times 10^9/l$ (range $0.01-0.15 \times 10^9/l$). To date, over 5000 of these units have been registered with the BBMR and Netcord. The analysis of ethnic origin of these CB units compared with bone marrow (BM) donors registered with the BBMR is indicated in Table II. It was evident that there was a higher proportion of UK ethnic minority donors amongst CB units than in the BM donors.

Units issued for transplantation

By July 2003, 59 units had been issued for transplantation; two in 1998, 15 in 1999, 18 in 2000, 10 in 2001, 10 in 2002 and the remaining four units in 2003. Of these, 41 (69%) were of European Caucasoid origin, nine (15%) were from the Indian sub-continent, two (3·3%) from Black donors, one

Table I. Volume and characteristics of cord blood units stored at the London Cord Blood Bank.

	Volume (ml)	TNC $\times 10^9$/l	WCC $\times 10^9$/l	CD34+ $\times 10^9$/l	NC viability (%)
Mean	75·8	12·5	10·4	0·04	95·2
SD	23·0	18·3	12·8	0·12	2·5
Median	71·9	11·9	9·9	0·03	95·6
Min	40·0	10·0	7·5	0·01	82·4
Max	240·2	24·8	8·5	0·15	99·1

NC, nucleated cells; TNC, total nucleated cells; WCC, white cell count (excluding nucleated red cells).

CD34 data was available for 1654 units only.

Table II. Ethnic distribution of cord blood and bone marrrow (BM) donors registered with the British Bone Marrow Registry (BBMR).

Ethnic origin	Cord Blood units ($n = 5070$)*	Bone marrow donors ($n = 92550$)*
European Caucasoid	57·7%	97·5%
Non-European Caucasoid	20·9%	0·7%
Black	6·2%	0·7%
Oriental	1·0%	0·1%
Other	1·7%	0·6%
Mixed	7·8%	0·5%
Unknown	4·7%	–

*Currently 51% of the total number of BM donor volunteers registered. Information unavailable for the remaining 49% (data not published).

(1·7%) of Oriental origin and the remaining 6 (10%) were of mixed ethnic origin. These CB units were issued for transplantation to 59 patients in a variety of countries; 17 to the UK, 26 to other European countries, nine to the USA, four to Australia, two to South America and one to Hong Kong. Forty-eight transplants were reported to the Eurocord registry. Four units were either expanded in vitro or co-infused with other units and were excluded from this clinical analysis.

Transplant outcome

Follow-up data from 44 transplants performed in 12 countries and 25 transplant centres between January 1998 and March 2003 were analysed. The median follow-up of survivors was 14 months (range, 3–44).

Patient characteristics

Patients were aged between 0·7 and 47 years with a median of 8 years. The average patient weight was 28 kg (range 5–80 kg). The majority of patients had malignancies; 23 (52%) had acute leukaemia, with 12 in advanced stage (>2 patients in complete remission or in non-remission) of the disease and seven with Chronic Myeloid Leukaemia (CML). Other malignancies included Myelodysplastic Syndrome [MDS, $n = 1$) and non-Hodgkin's Lymphoma (NHL, $n = 3$) and 1]. The

remaining seven patients were suffering from bone marrow failure syndromes (Table III). Two patients had previously received an autologous bone marrow graft, and three patients had been transplanted earlier with allogeneic bone marrow stem cells. Unfortunately, very limited information was available on the ethnic origin of these patients as transplant centres in some countries were unable to provide this data because of legal restrictions.

Table III. Patient characteristics.

Characteristics	N (% or range) ($n = 44$)
Diagnosis	
ALL	15 (34%)
AML	8 (18%)
CML	7 (16%)
MDS	1 (2%)
NHL	3 (7%)
Fanconi anaemia	7 (16%)
PNH	2 (5%)
SAA	1 (2%)
Gender	
Male	32 (73%)
Female	12 (27%)
Gender match	
Yes	21 (50%)
No	21 (50%)
Age at transplantation	
<15 years	36 (82%)
≥15 years	18 (8%)
Median age, years	8 (0·7–47)
Median body weight, kg	28 (5–80)
Recipient's CMV serology	
Negative	24 (56%)
Positive	19 (44%)
ABO	
Compatible	18 (42%)
Minor incompatibility	12 (28%)
Major incompatibility	13 (30%)

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; PNH, paroxysmal nocturnal haemoglobinuria; SAA, severe aplastic anaemia.

Transplant characteristics: preparative regimens and GVHD prophylaxis

Seventeen patients received cyclophosphamide and total body irradiation alone or in combination with a second drug. Thirteen patients received a combination of busulphan and cyclophosphamide alone or in combination with a third drug, 11 were treated with a fludarabine-containing regimen and three patients received other regimens. Thirty-eight (86%) patients received some form of humoral immunotherapy before day 0, in addition to the conditioning (antithymocyte globulin/antilymphocyte globulin/monoclonal antibody).

Graft-versus-host disease prophylaxis consisted of Cyclosporine A (CsA) and prednisone in 57% of patients ($n = 25$). The remaining patients received CsA alone ($n = 7$), FK 506 plus another ($n = 5$), CSA plus methotrexate with or without prednisone ($n = 3$) or CsA and Mycophenolate Mofetil ($n = 3$).

CB characteristics and HLA matching

The median number of nucleated cells infused was 4×10^7/kg, with a range of $1.1–16.0 \times 10^7$/kg. The median number of CD34+ cells infused averaged 1.30×10^5/kg (range 0.20–4.90; data available in 29 patients only) with a median granulocyte-macrophage CFU (CFU-GM) of 2.9×10^4/kg (range 0.42–92.0; data available on 20 patients only). HLA matching was determined at the specificity level for HLA-A and HLA-B and allelic typing for DRB1 in 41 of the donor/recipient pairs. Of these, 6/6 HLA-matched transplants were performed in seven patients, 16 received a 5/6 match, a further 16, a 4/6 match and two patients a 3/6 match (Table IV).

Engraftment

Probability of neutrophil recovery at day 60 was $74 \pm 7\%$ (Fig 1). Two patients died at days +10, +21 and were censored at time of death. Eleven patients were considered as 'non-engraftment' at day 60: five patients had autologous reconstitution, four patients received a second transplant (three autologous and one allotransplant) before day 60, one patient died in aplasia after day 60 and one patient engrafted after day 60 and was considered as 'non-engraftment'. The median number of days (considering all patients) taken to reach a neutrophil count of at least 0.5×10^9/l was 28, with a range of 11–41 days). Average time to platelet recovery of 20×10^9/l was 56 days, ranging between 19 and 99 days).

In univariate analysis for neutrophil recovery, no significant effect of HLA match, patient age or cell dose on the probability of engraftment was observed. The estimated neutrophil recovery at day 60 was 70% for patients <15 years of age and 87% for patients aged ≥15 years ($P = 0.19$). Similarly, it was 75% ($n = 21$) of those infused with $< 3.7 \times 10^7$ nucleated cells/kg compared with 74% ($n = 16$) for patients receiving a higher cell dose ($P = 0.82$).

Table IV. Characteristics of the transplanted CB units.

Variable	Number (%)
No. of HLA mismatches ($n = 41$)*	
0	7 (17%
1	16 (39%)
2	16 (39%)
3	2(5%)
Details of HLA mismatches ($n = 41$)*	
1 mismatch*	
1A	2 (5%)
1B	5 (12%)
1DR	9 (22%)
2 mismatches*	
1A & 1B	7 (17%)
1B & 1DR	5 (12%)
1A & 1DR	3 (7%)
2A	1 (2%)
3mismatches*	
1B & 2DR	1 (2%)
2B & 1DR	1 (2%)
Total nucleated cells infused ($n = 37$)	
Median	4×10^7/kg
Range	$1.1–16.0 \times 10^7$/kg
CFU-GM ($n = 20$)	
Median	2.9×10^4/kg
Range	$0.4–92 \times 10^4$/kg
CD34 cells infused ($n = 29$)	
Median	1.3×10^5/kg
Range	$0.2–4.9 \times 10^5$/kg

*Definition for HLA-A & B is at specificity level and for HLA-DRB1 at the allelic level. DRB1 data missing on three patients.

GVHD

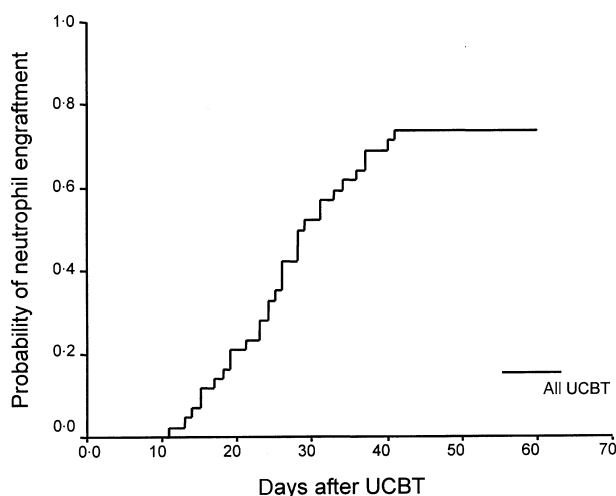Using the Kaplan-Meier method, the probability of a patient having acute GVHD (grade II or more) at day 100 was

Fig 1. Probability of neutrophil recovery of $≥0.5 \times 10^9$/l at day 60 in 44 patients transplanted with cord blood between January 1998 and March 2003. UCBT, umbilical cord blood transplant.

$37 \pm 7\%$. In 27 patients (62%) acute GVHD was absent or grade I, in eight (18%) grade II, in four (9%) grade III and in five (11%) grade IV. The likelihood of chronic GVHD at 2 years was estimated as 21%, with five of 25 patients at risk.

Survival, disease-free survival and relapse

The Kaplan-Meier estimate of overall survival at 2 years was $49 \pm 8\%$ (Fig 2). It was $49 \pm 9\%$ for patients transplanted with malignant disorders and $50 \pm 16\%$ for patients transplanted with non-malignant diseases ($P = 0.89$) (data not shown). We did not find an association of cell dose with survival in this analysis. However, those recipients <15 years of age were more likely to survive than older patients, with $58 \pm 9\%$ survival compared with $13 \pm 12\%$ respectively ($P = 0.008$). There was also an observed effect of recipient CMV status, with CMV negative patients having a better prognosis than those who were CMV positive ($P = 0.02$). All our donations issued for transplant were CMV negative. In addition, ABO-mismatched patients had a lower probability of survival i.e. $23 \pm 12\%$ compared with $67 \pm 9\%$ of those who were compatible or had minor ABO incompatibility, ($P = 0.008$). Two years after transplantation the survival rate was 69% and 54% for those patients receiving a 6/6 HLA-matched or 5/6 HLA-matched CB unit, respectively. It was 38% in those patients who were mismatched for two HLA antigens. Two patients transplanted with three HLA disparities are alive. In a multivariate analysis for survival, three factors were associated with death: i) recipient's positive CMV serology prior to CB transplant (CBT) [Hazard ratio (HR): 4·08; 95% Confidence Interval (95% CI): 1·49–11·17; $P = 0.006$]; ii) Major ABO incompatibility (HR: 2·18; 95% CI: 1·34–3·53; $P = 0.02$); and iii) adults (HR: 3·60; 95% CI: 1·36–9·57; $P = 0.01$).

Disease-free survival at 2 years was $41 \pm 8\%$ (Fig 3). Seven patients with malignant disorders relapsed [four acute lymphoblastic leukaemia (ALL), 1 acute myeloid leukaemia (AML), 1 NHL and 1 MDS] and relapse incidence at 2 years for patients with malignant diseases was $29 \pm 10\%$. No patient with bone marrow failure syndrome had late graft failure.

Cause of death

Twenty-one of the 44 patients died. Relapse or disease progression occurred in four patients (19%). Other transplant-related causes of death included seven infections and non-engraftment, haemorrhage ($n = 2$), acute respiratory distress syndrome ($n = 2$), cardiac toxicity ($n = 1$) and GVHD ($n = 3$, 10%). The remaining two patients died from other causes.

Discussion

The LCBB has been storing CB units following a standard, validated procedure for 7 years from hospitals targeted for their ethnically diverse populations, resulting in a Cord Bank with nearly half of the units originating from ethnic minorities within the UK. So far, 31% of CB units issued for transplant were of non-European Caucasoid origin, thereby fulfilling one of the LCBB's primary aims. Interestingly, this approach has been fully validated in the recent report by Wagner *et al* (2002), which suggested that CB banks should focus not only on collecting units of larger volume but also on the collection of CB from ethnic minorities.
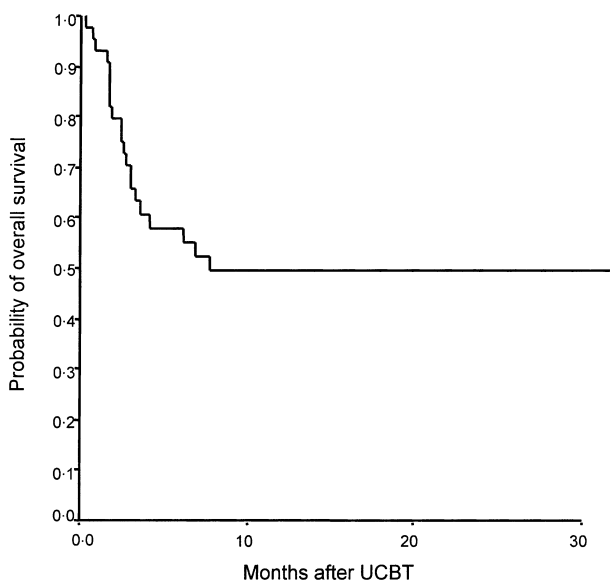

Fig 2. The Kaplan-Meier estimate of overall survival at 2 years in 44 patients transplanted with cord blood between January 1998 and March 2003; median follow-up of 14 months (range 3–44). UCBT, umbilical cord blood transplant.
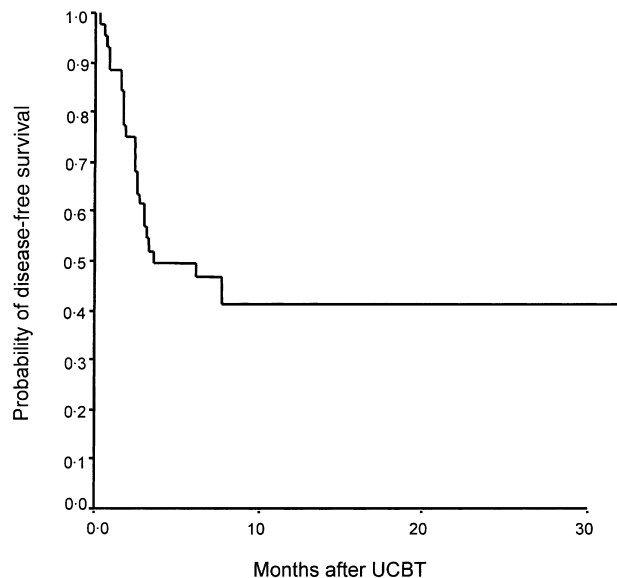

Fig 3. The Kaplan-Meier estimate of disease-free survival at 2 years in 44 patients transplanted with cord blood between January 1998 and March 2003; median follow-up of 14 months (range 3–44). UCBT, umbilical cord blood transplant.

Since 1998 the LCBB has provided CB units for transplantation into children and adult patients. The average number of cells infused was above the suggested threshold of 3.7×10^7/kg (Gluckman & Locatelli, 2000). The probability of neutrophil engraftment was 74% at day 60; this included adult patients who formerly would have been perceived as unsuitable recipients of CB because of low cell dose, thus confirming recent publications reporting favourable outcome in some adults following CB transplantation (Gluckman *et al*, 2001; Laughlin *et al*, 2001; Sanz & Sanz, 2002). Patient age did appear to have some effect on overall survival, with those patients <15 years of age more likely to be alive at 2 years compared with the older cohort. This may partly be explained by the possibility that adults transplanted with CB are higher-risk patients, for whom it has not been possible to identify a bone marrow donor, because of lack of time or availability of compatible donors in the bone marrow registries.

In our study, the degree of HLA matching of donors and recipients was substantially lower than that recommended for bone marrow transplantation (Hansen *et al*, 2000). However, although the numbers reported in this study were too small to be statistically significant, there appeared to be no major difference in the survival of those patients with a single HLA mismatch compared with those receiving a 6/6 HLA match. This data thus support previous reports, where HLA mismatching appears to be better tolerated in CB transplantation compared with BM grafts (Barker *et al*, 2001; Rocha *et al*, 2001). Recently, Eurocord and other groups have stated that the outcome of umbilical CBT become poorer with more than two HLA disparities, based on serological typing for class I and high resolution typing for class II (Gluckman *et al*, 2001; Wagner *et al*, 2002). At present, no data are available to establish the possible numbers of mismatches using high resolution molecular typing of class I and II alleles. As with other studies, the small numbers prevented the analysis of the significance of matching for other HLA loci e.g. HLA-Cw or DQ, which may account for the variation in outcome of patients with the same level of HLA-A, -B and -DR matching. The number of transplants analysed was also insufficient to examine the impact of HLA matching on frequency and severity of GVHD. However, the levels of GVHD observed were similar to those reported by others (Thomson *et al*, 2000; Rocha *et al*, 2001; Wagner *et al*, 2002), suggesting a lower risk of GVHD following infusion of HLA-mismatched CB compared with HLA-matched BM transplantation. These results are particularly important when considering the high-risk, heterogenous group of patients included in this study, including 18 patients >15 years.

Our results also indicate that infection was the main cause of transplant-related mortality, which may in part be explained by the delayed engraftment, compared with the rate of engraftment reported following BM and peripheral blood stem cell transplants (Korbling & Anderlini, 2001). However, the rate of relapse compared favourably with previously reported data, resulting in the death of only 4/34 patients with some form of malignancy. However, there was insufficient data to draw any meaningful conclusions from this, although it appears to refute the perception that CB transplantation carries a higher risk of relapse. The immunological reconstitution of CB haemopoietic stem cells is not well documented and more studies are required, particularly regarding the comparison with BM transplantation, before definitive conclusions can be drawn.

In summary, this single centre has provided 59 CB units for transplantation into a high-risk, heterogeneous group of patients, including both adults and children, with overall survival comparable with previously published results (Rubinstein *et al*, 1998; Gluckman, 2000; Wagner *et al*, 2002). Where required, the units were provided within a few days of request. Of the 44 transplants analysed, 23 recipients are still alive, thus confirming that CB is a good alternative source of haemopoietic stem cells for patients requiring a transplant but who do not have a suitable related or unrelated BM donor.

Acknowledgments

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